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# QUANTITATIVE-GENETIC ANALYSIS OF NITROGEN CONTENT IN THE GRAINS OF COMMON WHEAT CROSSES

by

© PAUL BANKS

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Quantitative-Genetic Analysis of Nitrogen Content in the Grains of Common Wheat crosses submitted by Paul Banks in partial fulfilment of the requirements for the degree of Master of Science.

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## ABSTRACT

Four common wheat ( Triticum aestivum L. ) cultivars, two each from Canada and Mexico, were reciprocally crossed and backcrossed in all possible combinations. The seeds obtained from different generations were analyzed for nitrogen content using standard micro-kjeldahl techniques. The genetic study of this quantitative character utilized Griffing's ( 1956b ) method of analysis for general combining ability, specific combining ability and reciprocal effects and Aksel's ( 1974 ) method of analysis of means of reciprocal crosses. The results of the general and specific combining ability analysis show that there are genetic differences between the cultivars for seed nitrogen content and that additive gene effects are more important than non-additive gene effects in their contribution to the variance. The analysis of means has shown that the response of the idiotype in different environments was cross dependent and that more than one genetic model was required to explain the data. Plasmatype affected the expression of paternal alleles in five of the six crosses considered and alleles for reduction of nitrogen content were found to be mostly dominant.

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## INTRODUCTION

The character nitrogen, and implicitly, protein content in the grains of cereals belongs to that group of characters, some of economic value, which show a continuous gradation of expression within certain developmental-physiologically and genetically determined limits and are referred to as quantitative characters. In regard to a quantitative character in cereals and in plants generally, the notion of phenotype can be derived as a function of variables idiotype and environment, which are themselves functions of variables genotype and plasmatype, and of different climactic and edaphic factors respectively. In other words, the term phenotype applied to a quantitative character quite often may imply a very complex situation. This complexity, often increased by associations among characters some of which may not be desirable, complicates the task of plant breeders in obtaining superior cultivars and that of geneticists in elucidating the mode of transmission, expression and association of quantitative traits. Since in the case of such traits, the individuals of a population cannot be grouped by their phenotypes into a few discrete genotypic groups, biometrical methods are used in the study of the genetics of such characters. The purpose of the present work is to study the inheritance of nitrogen content in the grains of common wheat ( Triticum aestivum L. ) by using a set of reciprocal crosses and backcrosses obtained from four different parental cultivars.

## LITERATURE REVIEW

Experiments conducted by different researchers at different times and in different places have shown that protein content of the grains of cereals is highly susceptible to changes in different environmental factors ( Botkin 1935, Heller & Sieglinger 1944, Eck & Stewart 1954, Williams & Smith 1954, Genter et al 1956, Miller et al 1964, Campbell & Pickett 1968, Hojjati & Maleki 1972 ). Moreover, with regard to protein content, it has been noted that the contribution of environmental factors to the phenotypic variation was usually larger than that of genotypic differences among cultivars ( Bhatty 1963 ). Several studies conducted on different cereal crops have reported negative correlation for the characters yield of grain and protein content in the grains in response to environmental changes ( Botkin 1935, Hobbs 1953, Eck & Stewart 1954, Miller et al 1964 ). There are also reports for negative correlation between protein content and lysine content expressed per weight of protein ( Sauberlich et al 1953, Gunhardt & McGinnis 1957, Bhatty et al 1963 ). However, it has been claimed that for wheat this correlation is found only in samples with less than 13.5% protein ( Hojjati & Maleki 1972 ). In studies conducted by varying the genotype, i.e., by using lines of cereal crops differing for grain yield, it was found that the amount of protein in the grains was negatively associated with the yield of grain ( East & Jones 1920, Clark & Quisenberry 1929, Malm 1968, Collins & Pickett 1972a,b ). However the coefficients of correlation, although significant, were not high enough to suggest pleiotropic gene effect instead of linkage.

Frey ( 1949 ) has studied the inheritance of protein and zein content in the grain of corn ( maize ) by crossing high and low protein lines and analyzing their progeny. From his studies, he has concluded that the cross fits an arithmetic gene interaction model ( Charles & Smith 1939 ) and that there are at least 22 genes affecting the character protein content and at least 6 genes affecting the character zein content. However, he has assumed that the protein content of seeds borne on an F1 plant are a character of that generation which is not true.

Johnson and Aksel ( 1964 ) have studied, among others, the inheritance of nitrogen content in barley grains by using a twelve-parent diallel cross. They found the character to be inherited as a dominant trait with high expression of the character associated with recessive genes.

Experimental results on protein content in grains of crosses between common wheat lines were reported by Clark and Quisenberry ( 1929 ). However, the interpretation of their results is rather difficult since the generations considered were not grown in the same year. Aksel ( 1956 ) has investigated the protein content in the grains of twelve common wheat cultivars and ten of their crosses. He has found that in two F1 crosses the protein content of the grain was the same as, and in eight lower than, that of the lower parent. The same was true for groups of plants taken from the F2 generation of some of the crosses. For others the amount of protein in the grains of individual F2 plants was either intermediate, the same as or higher than that of the higher parent.

## MATERIALS AND METHODS

### Materials

The materials used in this study were obtained from five supposedly idiotypically different cultivars of hexaploid wheat ( Triticum aestivum L.) which were crossed and backcrossed in all possible combinations ( Soomro 1974 ). The cultivars used and their provenances are:

1) M-Marquis - Canadian
2) CH-Chinook - Canadian
3) I-Inia - Mexican
4) C-Ciano - Mexican
5) K-Khush-hal - Pakistani

There are phenotypic differences between the parental lines for the character plant height and for characters determining the spike and kernel morphology. On the other hand, some genetic similarity is to be expected between the Mexican varieties Inia and Ciano since they were derived from crosses which involved the same cultivar Sonora 64.

For each pair of cultivars the following generations of reciprocal crosses were grown ( the first parent indicated is the female ):

| | | |
|---|---|---|
| [AB]F1 | [BA]F1 | - reciprocal F1s |
| [AB]F2 | [BA]F2 | - selfed reciprocal F1s |
| [(AB)A]F1 | [(BA)A]F1 | reciprocal backcrosses |
| [(AB)B]F1 | [(BA)B]F1 | |
| [A(AB)]F1 | [A(BA)]F1 | |
| [B(AB)]F1 | [B(BA)]F1 | |
| [(AB)A]F2 | [(BA)A]F2 | selfed reciprocal backcrosses |
| [(AB)B]F2 | [(BA)B]F2 | |
| [A(AB)]F2 | [A(BA)]F2 | |
| [B(AB)]F2 | [B(BA)]F2 | |

The notation follows that of Aksel ( 1974 ).

The five parentals, ten different pairwise combinations of these five parentals and twenty different crosses for each of these ten different combinations provide a total of 205 genetically different families. The F2 generation was duplicated in each replication since it has the largest number of segregating loci, to give a total of 225 plots per replication.

The materials were grown in 1972 at two different locations in or near Edmonton, Alberta; Parkland farm ( Department of Plant Science, University of Alberta ) and Ellerslie Field Laboratory ( Department of Genetics, University of Alberta ). Fertilizer was applied before seeding at a rate of 56 kg/ha of 11-55-0. The plots used were one-row-plots of 30 seeds each. The density of seeding was 30 cm between rows and 15 cm between seeds within rows. Seed harvested from these plants was used in the present quantitative genetic study of the nitrogen content of the flour of whole kernels.

One gram of seed was taken from each of ten consecutive plants in a row and pooled. In very few instances were there less than ten plants per row; when this was the case, an equal amount of seed was taken from each plant to total ten grams. This amount of seed is the minimum amount that can be ground in the mill used. In the case of F2 plants, a total of five grams of seed from each of the two rows in a replication was taken, again equally divided among the plants in a row. In the analysis of the five parental cultivars in the three different replications within each of the two locations, the ten gram samples of seed were treated separately. When all of the crosses of certain parental strains were studied, seed from the

two locations, ( Ellerslie and Parkland ), was maintained separate but the ten gram samples of each of the three replications within a location were pooled ( see Results section ).

Methods

For this study, chemical methods from the Official Methods of Analysis of the Association of Official Agricultural Chemists (A.O.A.C.) were modified to handle large numbers of samples with the apparatus available. These changes should not affect the genetic analysis since the relative differences in nitrogen content of different families will remain the same. What follows is an outline of the procedures used on flour from whole kernels of wheat.

The seed sample was ground for five minutes to a fairly uniform appearance in a water-cooled CRC micro-mill. 1.5 grams of flour was spread out in each of four aluminium weighing dishes and allowed to equilibrate in air for a period of time not less than four hours because of the hygroscopic nature of freshly ground wheat flour. Glass weighing dishes ( 30x50 mm borosilicate glass ) were dried in a 130°C oven for 1/2 hour and cooled in a dessicator over Drierite for 1/2 hour. Flour samples ( weighing approximately two grams ) were spread out in each pre-weighed glass dish, weighed ( to four decimal places ), dried for one hour at 130°C, closed while still in the oven and then cooled for 1/2 hour in a dessicator over Drierite before the final dry weight was determined. Dry weight determinations were performed in duplicate on each sample of seed being analyzed. Kjeldahl nitrogen determinations were performed in triplicate on each of the equilibrated samples.

0.1 grams of flour sample was taken from the aluminium pans after a minimum of four hours in air and was digested for one hour ( see below for time requirements ) in a 30 ml kjeldahl digestion

flask with the following additions ( all chemicals used were reagent grade ).

2-3 carbon boiling chips
1.34 grams of a catalyst mixture prepared by mixing 130 grams $K_2SO_4$ with 4.0 grams red HgO
2 ml concentrated $H_2SO_4$

The flour samples contained no large particles which were resistant to acid digestion. Following digestion, 8 ml of a sodium hydroxide/ sodium thiosulphate solution ( 50 grams NaOH and 5 grams $Na_2S_2O_3 \cdot 5H_2O$ ) were added to the sample in a Pregl type ( Parnas-Wagner ) micro-kjeldahl distillation apparatus. The ammonia released was steam-distilled from the sample and was trapped in 5 ml of a boric acid solution ( 4% $H_3BO_3$ ) in a 125 ml erlenmeyer flask. Steam distillation was continued until at least 15 ml of water had condensed in the flask. Three drops of a bromcresol green/ methyl red indicator were added to the distillate which was then titrated to the first appearance of pink using 0.02 N HCl.

Experiments were run on a pooled sample of seeds to determine the minimum time necessary for complete digestion of flour as measured from the time of addition of acid. Distillations of the samples were done at random for five replications each of one, two, three and four hours of digestion and for four replications of five hours of digestion according to the procedures already outlined. There was no significant difference among hours of digestion in the amount of ammonia released and there was no significant heterogeneity of replications within treatments. Therefore, all samples in this study were digested for one hour. An even shorter time of digestion, 40

minutes, has been used for corn protein with a different catalyst mixture ( Villegas & Mertz 1971 ).

Calculation of percent nitrogen

The percent nitrogen in the flour samples was calculated with the following formula:

$$\%N = \frac{(\text{ ml of 0.02 N HCl }) \times 14.007 \times (\text{ 100\% })}{(\text{ 100 mg flour }) \times (\text{ 1 - L/100 })}$$

where 14.007 is the equivalent weight of nitrogen and L is the mean of the two dry weight determinations expressed as percent water per air-dry flour. Determinations on blanks were not performed for each sample. Instead the pH of the water in the steam generator was adjusted with $H_3PO_3$ so that less than 0.1 ml of 0.02 N HCl was required to titrate a distilled water sample. The kjeldahl determinations ranged from 8.5 ml to 11.5 ml of 0.02 N HCl. Three figure accuracy has been assumed for all the percent nitrogen values which ranged from 2.70 to 3.40.

It is customary for nutritionists to multiply the percent nitrogen by 6.25 to convert it to crude protein since certain calculations important in nutrition studies are facilitated by this conversion ( Pomeranz 1971 ).This conversion factor is an overall average value and is used for all proteins. Cereal chemists multiply the percent nitrogen by 5.7 to estimate the percent crude protein but precise methods of amino acid analysis have shown that the proper conversion factor may vary from 5.24 to 5.68 depending on the wheat protein studied ( Pomeranz 1971 ). In this study, the percent nitrogen values have not been multiplied by a conversion factor for this is merely a uniform change in the scale of measurement. While the analysis has been performed on the character

nitrogen content, the results should apply to the character crude protein as well.

## RESULTS

### Exploratory Analysis

Six nitrogen determinations were performed on the five parentals for the six replications - three at Ellerslie ( ER ) and three at Parkland ( PR ) - and on the twenty possible F2 families for Ellerslie Replication - 1 ( ER-1 ). The F2 generation refers to the seed generation that was collected from F1 plants, not to the generation of the plants.

An analysis of variance was performed on data ( Appendix I ) for the five parental lines grown at the two locations. A fixed model was assumed and all terms were tested for signifigance with respect to the Error MS. The results are given in Table 1 and show that the parentals are genetically different for the character nitrogen content and that this character also depends on environment. The F ratio for the Location MS is much larger than the Between Replications within Locations MS. This shows that the difference between the effects at the two locations is much greater than that within locations. Because of this, it is reasonable to pool the seed samples from the three replications before performing the nitrogen determinations in all the crosses. Moreover, using larger samples should minimize genetic sampling errors which may be important since all of the non-parental generations are genetically segregating and since the character nitrogen content is, most likely, controlled by polygenes.

The F2 generations were studied in ER-1 in order to obtain an idea of the range of variation to be expected in the hybrid generations. Since it was not practicable to analyze all the

Table 1

Joint analysis of variance for the five parental lines grown at the two locations

| Source | SS | DF | MS | F |
|---|---|---|---|---|
| Total | 1.736170 | 29 | | |
| Cultivars | 1.207717 | 4 | 0.301929 | 53.44** |
| Locations | 0.341120 | 1 | 0.341120 | 60.38** |
| Locations x Cultivars | 0.010263 | 4 | 0.002566 | 0.45ns |
| Between Replications within Locations | 0.086676 | 4 | 0.021669 | 3.84* |
| Error | 0.090393 | 16 | 0.005650 | |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

combinations of the five parents, one parental line and its hybrid progenies were to be removed.

The preliminary analyses - shown in Table 3 - revealed a certain degree of non-equivalence of reciprocal crosses which was most marked in (MC)F2, (MI)F2 and (CCH)F2 crosses. The differences between reciprocal crosses involving K parent were small so it was decided to exclude K parent and its derivatives from further analyses. Although the mean values of the parentals in ER-1 differ from the mean values of the parentals averaged over the two locations ( Table 2 ) it was hoped that the pattern of non-equivalence would not depend upon environment and that ER-1 would be representative.

In all subsequent analyses, the four parentals M, C, I and CH and their hybrid families were used and seed samples from the three replications within each location were pooled. The families for the four parentals were randomized and three nitrogen determinations were made on the same day on each family. After this was completed, the families resulting from the cross of C and M were randomized and analyzed again. The lines M and C had, respectively, the lowest and the highest expressions of the character and their F2 generations showed the greatest non-equivalence of reciprocal crosses ( Tables 2,3 ).

Other families were reanalyzed if there were only two instead of three determinations or if the range in titration values was greater than 0.3 ml of 0.02 N HCl.

Table 2

The mean percent nitrogen content for the five parental lines averaged over the two locations

| Cultivar | mean %N over two locations | mean %N in ER-1 |
|---|---|---|
| M | 2.75 | 2.79 |
| K | 2.63 | 2.76 |
| C | 3.21 | 3.32 |
| I | 2.87 | 3.01 |
| CH | 2.88 | 2.88 |

Table 3

The mean percentage nitrogen content of the reciprocal F2 crosses in ER-1

| Parentals | (AB)F2 | (BA)F2 | (AB)F2-(BA)F2 |
|---|---|---|---|
| M,K | 2.79 | 2.74 | .05 |
| M,C | 3.06 | 2.79 | .27 |
| M,I | 2.87 | 2.75 | .12 |
| M,CH | 2.77 | 2.85 | -.08 |
| K,C | 2.79 | 2.80 | -.01 |
| K,I | 2.96 | -- | -- |
| K,CH | 2.75 | 2.68 | .07 |
| C,I | 2.88 | 2.93 | -.05 |
| C,CH | 2.90 | 3.02 | -.12 |
| I,CH | 3.03 | 3.01 | .02 |

General and Specific Combining Ability

The F2 and F3 generations were analyzed for family differences and location effects, and for general and specific combining ability ( g.c.a. and s.c.a. ) according to Griffing's ( 1956b ) method 1 model 1. The results are given in Tables 4,5 and 6 for F2 generation and in Tables 7,8 and 9 for F3 generation. The complete data are in Appendix II.

The analyses of variance for F2 ( Table 4 ) and F3 ( table 7 ) show that there are varietal differences and location effects on the character nitrogen content - the latter component being a more important source of variation than the former.

The g.c.a. and s.c.a. analyses of variance, for both F2 ( Table 5 ) and F3 ( Table 8 ), show significant g.c.a. and s.c.a. effects. On the other hand, neither generation shows significant reciprocal differences. If the estimates of g.c.a. in these materials measure predominately additive genetic variance ( Griffing 1956a ) then they should remain unaffected by changes in the degree of heterozygosity; if the estimates of s.c.a. measure predominately non-additive genetic variance, then they should be affected by the degree of heterozygosity. Consequently, the ratio of g.c.a. MS to s.c.a. MS should increase for successive selfed generations. The results are consistent with these expectations. First of all, pairwise comparisons ( Table 10 ) show that the g.c.a. estimates for the two generations are the same. Secondly, the s.c.a. estimates are larger for the F2 than for the F3 generation, especially the estimates on the diagonal ( compare Tables 6 and 9 ). Finally, the ratio of

F2 Generation

Table 4

two-way analysis of variance

| Source | SS | DF | MS | F |
|---|---|---|---|---|
| Families | 0.591201 | 15 | 0.039413 | 6.49** |
| Locations | 0.0722 | 1 | 0.0722 | 11.89** |
| Error | 0.091049 | 15 | 0.00607 | |

Table 5

analysis of variance for g.c.a., s.c.a. and reciprocal differences

| Source | SS | DF | MS | F |
|---|---|---|---|---|
| g.c.a. | 0.2188 | 3 | 0.072933 | 216.28** |
| s.c.a. | 0.070102 | 6 | 0.011684 | 11.55** |
| reciprocal differences | 0.006852 | 6 | 0.001142 | 1.13ns |
| error | | 15 | 0.001012[a] | |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

[a] this error MS is $\frac{\text{Error MS}}{bc}$ where b is the number of locations and c is the number of observations averaged for each entry

F2 Generation

Table 6

s.c.a. and g.c.a. values and reciprocal differences
( below the main diagonal )

| | s.c.a. | | | | g.c.a. | $\hat{\sigma}^2_{g_i}$ | $\hat{\sigma}^2_{s_i}$ |
|---|---|---|---|---|---|---|---|
| | M | C | I | CH | | | |
| M | $.054^{**}$ | $.018^{ns}$ | $-.026^{ns}$ | $-.046^{**}$ | $-.060^{**}$ | .0034 | .0013 |
| C | $.109^{**}$ | $.115^{**}$ | $-.055^{**}$ | $-.078^{**}$ | $.142^{**}$ | .0200 | .0045 |
| I | $.015^{ns}$ | $.020^{ns}$ | $.098^{**}$ | $-.017^{ns}$ | $-.031^{**}$ | .0008 | .0017 |
| CH | $.015^{ns}$ | $.015^{ns}$ | $.030^{ns}$ | $.140^{**}$ | $-.051^{**}$ | .0024 | .0037 |

$u = 2.869^{**}$

$sd(\hat{u}) = .0056$
$sd(\hat{g}_i) = .0069$
$sd(\hat{s}_{ii}) = .0169$
$sd(\hat{s}_{ij}) = .0126$
$sd(\hat{r}_{ij}) = .0220$

$sd(\hat{g}_i - \hat{g}_j) = .0112$
$sd(\hat{s}_{ii} - \hat{s}_{jj}) = .0225$
$sd(\hat{s}_{ii} - \hat{s}_{ij}) = .0251$
$sd(\hat{s}_{ii} - \hat{s}_{jk}) = .0195$
$sd(\hat{s}_{ij} - \hat{s}_{ik}) = .0195$
$sd(\hat{s}_{ij} - \hat{s}_{kl}) = .0159$

( ** ) = $P \leq 0.01$
( * ) = $0.01 < P \leq 0.05$
( ns ) = $P > 0.05$

F3 Generation

Table 7

two-way analysis of variance

| Source | SS | DF | MS | F |
|---|---|---|---|---|
| Families | 0.491570 | 15 | 0.032771 | 8.30** |
| Locations | 0.072390 | 1 | 0.072390 | 18.33** |
| Error | 0.055265 | 14 | 0.003948 | |

Table 8

analysis of variance for g.c.a., s.c.a. and reciprocal differences

| | | | | |
|---|---|---|---|---|
| g.c.a. | 0.213163 | 3 | 0.071054 | 107.98** |
| s.c.a. | 0.022648 | 6 | 0.003775 | 5.74** |
| reciprocal differences | 0.009522 | 6 | 0.001587 | 2.41ns |
| error | | 14 | 0.000658[a] | |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

[a] this error MS is $\frac{\text{Error MS}}{bc}$ where b is the number of locations and c is the number of observations averaged for each entry

F3 Generation

Table 9

s.c.a. and g.c.a. values, and reciprocal differences

( below the main diagonal )

| | s.c.a. | | | | g.c.a. | | |
|---|---|---|---|---|---|---|---|
| | M | C | I | CH | | $\hat{\sigma}^2_{g_i}$ | $\hat{\sigma}^2_{s_i}$ |
| M | $.026^{ns}$ | $-.038^{*}$ | $.030^{ns}$ | $-.018^{ns}$ | $-.072^{**}$ | .0049 | .0010 |
| C | $.061^{**}$ | $.070^{**}$ | $-.043^{**}$ | $.011^{ns}$ | $.138^{**}$ | .0188 | .0014 |
| I | $.040^{*}$ | $.020^{ns}$ | $.053^{*}$ | $-.041^{*}$ | $-.035^{**}$ | .0010 | .0019 |
| CH | $.100^{**}$ | $.035^{ns}$ | $.049^{*}$ | $.048^{*}$ | $-.032^{**}$ | .0008 | .0007 |

$\hat{u} = 2.921^{**}$

$sd(\hat{u}) = .0064$

$sd(\hat{g}_i) = .0079$

$sd(\hat{s}_{ii}) = .0192$

$sd(\hat{s}_{ij}) = .0143$

$sd(\hat{r}_{ij}) = .0180$

$sd(\hat{g}_i - \hat{g}_j) = .0128$

$sd(\hat{s}_{ii} - \hat{s}_{jj}) = .0257$

$sd(\hat{s}_{ii} - \hat{s}_{ij}) = .0287$

$sd(\hat{s}_{ii} - \hat{s}_{jk}) = .0222$

$sd(\hat{s}_{ij} - \hat{s}_{ik}) = .0222$

$sd(\hat{s}_{ij} - \hat{s}_{kl}) = .0181$

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

Table 10

Pairwise comparison of g.c.a. estimates from F2 and F3

| | F2 | F3 | difference F2 - F3 | sd | t |
|---|---|---|---|---|---|
| M | -.060 | -.072 | .012 | .0911 | $.13^{ns}$ |
| C | .142 | .138 | .004 | .1970 | $.02^{ns}$ |
| I | -.031 | -.035 | .004 | .0424 | $.09^{ns}$ |
| CH | -.051 | -.032 | -.019 | .0566 | $.34^{ns}$ |

g.c.a. MS to s.c.a. MS is 6.24 in the F2 and 18.8 in the F3 generation ( compare Tables 5 and 8 ).

The s.c.a. terms for the parentals are large in magnitude and positive in sign while the terms for the hybrids are small and of either sign. The high nitrogen content of the C parent is reflected in the high values of both g.c.a. and s.c.a.. M and CH parents, although with similar g.c.a., differ in nitrogen content because M has a low s.c.a. while CH has a high s.c.a.. This difference is quite marked in F2 and also persists in the F3 generation.

In none of the F2 or F3 crosses is the nitrogen content as high as that of the better parent. This is reflected in the many negative s.c.a. values found on the off-diagonal of the s.c.a. matrices. Furthermore, all of the families of crosses except those of parents M and C, show negative heterosis in at least one generation at one of the locations, and one set of them, the crosses of parents I and CH, shows negative heterosis in both generations at both locations.

Since the mean square due to reciprocal differences is not significant ( P = 0.05 ) for either the F2 or F3 generation of the diallel cross, it would seem that there are no plasmatic differences among the parentals. However, in the analysis of means that follows, all the parentals except C and I can be assumed to differ in plasmatype. There are three reasons for making this assumption despite the fact that the mean square due to reciprocal differences is not significant. Firstly, for all crosses except C,I and C,CH, the reciprocal differences observed in the F2 and/or F3 generations are significant ( Tables 6 and 9 ). Secondly, the analysis of means includes backcross generations

which show plasmatic effects in all crosses except the C,I cross, and thirdly the family means are not averaged for the two locations for the analysis of means as they were for the families used in the g.c.a. and s.c.a. analysis.

Analysis of means

In Aksel's ( 1974 ) notation, the idiotypes of the parents A and B are $I(A) = \{\P(A), U_k\{a\}_k\}$ and $I(B) = \{\P(B), U_k\{b\}_k\}$ and those of the $n^{th}$ generation of their reciprocal crosses are $I(AB)F_n = \{\P(A), U_k\{a,b\}_k\}$ and $I(BA)F_n = \{\P(B), U_k\{a,b\}_k\}$. It is assumed that the hybrid has the same plasmatype ( $\P$ ) as its female parent but that $\P(A) = \P(B)$ or $\P(A) \neq \P(B)$.

Non-equivalence of the reciprocal crosses with respect to the character expression implies that $\P(A) \neq \P(B)$ and conceivably may be due to the $\P(A)$ sensitivity of $U_k\{b\}_k \subset \{\P(A), U_k\{a,b\}_k\}$ or to the $\P(B)$ sensitivity of $U_k\{a\}_k \subset \{\P(B), U_k\{a,b\}_k\}$ or both. According to Aksel ( 1974 ) this idiotypic notation, in its most generalized form, is the same for crosses as well as for backcrosses.

The formulation of the genetic models and the analysis of sets of family means follows the pattern discussed by Aksel ( 1974 ) where three basic models are implied.

1) Genotypically and plasmatypically different parents:

$$E\bar{x}(XY)F_n = m + p[d] + q([h] \pm \Delta_{[h]}) \bar{+} r([d] \pm \Delta_{[d]})$$

where $E\bar{x}(XY)F_n$ denotes the expected ( E ) mean ( $\bar{x}$ ) of the $n^{th}$ ( $n = 1,2,3,\ldots$ ) filial generation of the cross between $X = ♀$ and $Y = ♂$ ( $X \neq Y$; $X = A,B,(AB),(BA)$); and $Y = A,B,(AB),(BA)$; p,q and r are coefficients such that $p + q + r = 1$ if $X = A$, $Y = B$ or $X = B$, $Y = A$; m is the mid-parental value, [d] is the sum of the genotypic values of differential loci when homozygous, [h] is the sum of the genotypic values of differential loci when heterozygous, and $\Delta_{[d]}$ and

$\Delta_{[h]}$ are the effects of maternal plasmatype on the expressivity of the paternal set of alleles when homozygous and heterozygous respectively.

2) Genotypically different but plasmatypically the same parents:

$$\bar{Ex}(XY)F_n = m \pm (p-r)[d] + q([h] \pm \delta_{[h]}) \mp r\delta_{[d]}$$

where $\delta_{[d]}$ and $\delta_{[h]}$ appear only in the backcrosses and denote gene dosis effects in homozygous and heterozygous states respectively.

3) A mixed model with both $\Delta$ and $\delta$ terms ( where, however, $\Delta$ may or may not have the same meaning as in model 1.

The separation of $\Delta_{[h]}$ from [h], or of both $\Delta_{[h]}$ and $\Delta_{[d]}$ from [h] and [d] is possible only if a certain combination of assumptions is imposed on the model and, of course, if the model fits the experimental data.

The expression of plasmatic effects, if any, in the crosses dealt with in this study is rather confusing and it does not appear to be consistant over environments and/or over generations. M,C cross shows patroclinous inheritance at Ellerslie but matroclinous inheritance at Parkland: M,I cross shows larger patroclinous effects in F2 than in F3 at Ellerslie but this is reversed at Parkland. After examination of the data for non-equivalence of reciprocal crosses, it was assumed that all crosses, except C,I, showed plasmatic effects and the reciprocal cross and backcross family means were separated into two supposedly plasmatically different groups.

Several of the backcross generations, in the ¶(A) group for instance, are genotypically the same; viz. $[A(AB)]F_n$, $[A(BA)]F_n$ and $[(AB)A]F_n$. The families were analyzed first with these backcrosses

taken separately and then with these families pooled. In most cases ( specific exceptions will be noted later ) this pooling resulted in lower standard errors for the parameters. Presumably, pooling these equations eliminates random variation among the backcross generations which cause lack of fit for the separate equations.

The $\Delta_{[h]}$ parameter can be separated from [h] only if the reciprocal F1 crosses are equivalent and certain assumptions are met with regard to the expression of gene effects ( see Aksel 1974, Smith & Aksel 1974 ). The F1 generation is not available in this study. Thus the term [h] in the additive-dominance model is in fact ($[h]\pm\Delta_{[h]}$) when used for families with the same plasmatype. Attempts were made to separate $\Delta_{[d]}$ from [d] but the matrix resulting from the families available could not be inverted without making certain assumptions as to the effect of the plasmatype on the parental gene set. For example, if the effect of the plasmatype on the expression of the character is the same for genes from the paternal gene set in homozygous or heterozygous condition then $(1/2)\Delta_{[d]} = \Delta_{[h]}$. If one also assumes ( assumption 6c, Aksel 1974 ) that the paternal gene set is unaffected by maternal plasmatype unless it has been associated with its maternal allelic set for at least one generation, then an invertible matrix results. This method has been tried for the M,C cross whose hybrid generations show the greatest non-equivalence of the reciprocals but no improved fit was obtained. This would imply that either the genetic assumptions necessary to invert the matrix are not valid or that the situation is complicated by epistasis.

In the additive dominance model used for one plasmatype,

[d] is in fact ($[d]\pm\Delta_{[d]}$).

The parameters ($[d]\pm\Delta_{[d]}$) and ($[h]\pm\Delta_{[h]}$) are measured with respect to $m' = [\bar{x}\{\P(A),U_k\{a\}_k\} + \bar{x}\{\P(A),U_k\{b\}_k\}]$ not with respect to $m = [\bar{x}\{\P(A),U_k\{a\}_k\} + \bar{x}\{\P(B),U_k\{b\}_k\}]$ as is the case for analyses where no plasmatic differences are assumed. If the families available for the different plasmatypes would permit $\Delta_{[d]}$ and $\Delta_{[h]}$ to be estimated, then the terms [d] and [h] would be measured from the mid-parental value m.

In all of these analyses, it has been assumed that there is no interaction between loci requiring the introduction of the parameters [i], [j] and [l] in place of $\delta_{[d]}$ and $\delta_{[h]}$ and/or $\Delta_{[d]}$ and $\Delta_{[h]}$. In point of fact, analyses were attempted using these parameters but the standard errors of the parameters were much larger than those obtained using $\delta_{[d]}$ and $\delta_{[h]}$ so the results have not been included. No attempt was made to combine these parameters in analyzing the data.

The systems of linear equations resulting from a particular model were solved by multiple regression methods ( Draper & Smith 1966 ).

The six crosses have been analyzed separately and the results are given is sequel. In the discussion that follows, [d]' and [h]' are ($[d]\pm\Delta_{[d]}$) and ($[h]\pm\Delta_{[h]}$) respectively.

Table 11

a) Mean nitrogen content of the families having Ciano ( C ) and Inia ( I ) as parents

| ¶( C ) families | ER | PR | ¶( I ) families | ER | PR |
|---|---|---|---|---|---|
| C parent | 3.39 | 3.15 | I parent | 2.98 | 2.83 |
| (CI)F2 | 2.93 | 2.90 | (IC)F2 | 2.93 | 2.94 |
| (CI)F3 | 3.02 | 2.96 | (IC)F3 | 2.98 | 2.97 |
| [C(CI)]F2 | 3.21 | 3.09 | [I(IC)]F2 | 2.84 | 2.88 |
| [C(CI)]F3 | 3.18 | 3.03 | [I(IC)]F3 | 2.94 | 2.88 |
| [C(IC)]F2 | 3.16 | 2.99 | [I(CI)]F2 | 2.97 | -- |
| [C(IC)]F3 | 2.96 | 2.95 | [I(CI)]F3 | 3.13 | 2.97 |
| [(CI)C]F2 | -- | 3.11 | [(IC)I]F2 | 2.98 | 2.88 |
| [(CI)C]F3 | 3.21 | 3.06 | [(IC)I]F3 | 3.08 | 2.99 |
| [(CI)I]F2 | 3.12 | 3.01 | [(IC)C]F2 | 3.18 | 3.05 |
| [(CI)I]F3 | 3.02 | 2.97 | [(IC)C]F3 | 3.03 | 2.90 |

b) Parameter estimates

| Parameters | ER | PR |
|---|---|---|
| $\hat{m}$ | $3.17^{**} \pm .03$ | $2.99^{**} \pm .02$ |
| $[\hat{d}]$ | $.21^{*} \pm .04$ | $.16^{**} \pm .02$ |
| $[\hat{h}]$ | $-.48^{*} \pm .12$ | $-.11^{ns} \pm .06$ |
| $\hat{\delta}_{[d]}$ | $-.57^{ns} \pm .33$ | $-.44^{ns} \pm .16$ |
| $\hat{\delta}_{[h]}$ | $.28^{ns} \pm .26$ | $.18^{ns} \pm .12$ |
| $R^2$ | .95 | .97 |
| DF | 3 | 3 |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

C,I Cross

The family means for this cross and the parameters estimated from them on the assumption of validity of model 2 are given in Table 11.

The significant [d] terms at both locations and the significant [h] term at Ellerslie show that the two parentals are genotypically different for the character nitrogen content. The alleles for lower nitrogen content appear to be mostly dominant.

While the additive effects of the loci affecting the character are the same in both locations ( $[\hat{d}]ER-[\hat{d}]PR = .05^{ns} \pm .04$ ) the dominance expressed by the alleles for lower nitrogen content depends upon environment ( $[\hat{h}]ER-[\hat{h}]PR = -.37^{**} \pm .13$ ). This implies genotype-environment interaction affecting the expression of heterozygous but not homozygous loci.

The $\delta_{[d]}$ terms, while not significant, show the same trend at both locations. This would suggest that gene dosis reduces the expression of the alleles affecting the character nitrogen content when these alleles are homozygous.

Table 12

a) Mean nitrogen content of the families having Marquis ( M ) and Ciano ( C ) as parents

| ¶( M ) families | ER | PR | ¶( C ) families | ER | PR |
|---|---|---|---|---|---|
| M parent | 2.85 | 2.73 | C parent | 3.39 | 3.15 |
| (MC)F2 | 3.15 | 2.93 | (CM)F2 | 2.89 | 2.94 |
| (MC)F3 | 3.05 | 2.78 | (CM)F3 | -- | 2.94 |
| [M(MC)]F2 | 3.08 | 2.95 | [C(CM)]F2 | 2.97 | 2.86 |
| [M(MC)]F3 | 2.91 | 2.84 | [C(CM)]F3 | 3.12 | 3.09 |
| [M(CM)]F2 | 2.89 | 2.74 | [C(MC)]F2 | 2.94 | 3.00 |
| [M(CM)]F3 | -- | 2.91 | [C(MC)]F3 | 2.99 | 2.94 |
| [(MC)M]F2 | 3.00 | 2.96 | [(CM)C]F2 | 3.10 | 2.91 |
| [(MC)M]F3 | 2.81 | 2.85 | [(CM)C]F3 | 2.99 | 2.96 |
| [(MC)C]F2 | 2.99 | 2.90 | [(CM)M]F2 | 2.79 | 2.75 |
| [(MC)C]F3 | 3.08 | 2.90 | [(CM)M]F3 | 2.86 | 2.81 |

b) Parameter estimates

| Parameters | ER ¶(M) | ER ¶(C) | PR ¶(M) | PR ¶(C) |
|---|---|---|---|---|
| $\hat{m}'$ | $2.90^{**} \pm .04$ | $2.95^{**} \pm .03$ | $2.82^{**} \pm .05$ | $2.90^{**} \pm .05$ |
| $[\hat{d}]'$ | $.05^{ns} \pm .05$ | $.44^{ns} \pm .05$ | $.06^{ns} \pm .05$ | $.22^{*} \pm .05$ |
| $[\hat{h}]'$ | $.48^{ns} \pm .13$ | $-.15^{ns} \pm .11$ | $.21^{ns} \pm .17$ | $0^{ns} \pm .17$ |
| $\hat{\delta}_{[d]}$ | $.78^{ns} \pm .27$ | $-.62^{ns} \pm .24$ | --- | --- |
| $\hat{\delta}_{[h]}$ | $-.49^{ns} \pm .20$ | $-.15^{ns} \pm .18$ | --- | --- |
| $R^2$ | .96 | .99 | .55 | .86 |
| DF | 2 | 1 | 4 | 4 |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

M,C Cross

Model 2 has been used in the analysis of the derivatives of this cross with the redefined m, [d] and [h] parameters; $\delta_{[d]} = \delta_{[h]} = 0$ has been assumed for Parkland data. The family means for this cross and the estimates of pertinent parameters are given in Table 12.

At Ellerslie, the m' value in ¶(M) is not significantly different from $\bar{x}$(M) and the [d]' term is small. Since $([d] \pm \Delta_{[d]}) = 0$, $\Delta_{[d]} = -[d]$ which implies that the expressivity of the additive effects of the $U_k\{c\}_k$ gene set are lost to the extent that it becomes indistinguishible from $U_k\{m\}_k$ gene set. Except for m', none of the parameters reach the conventional level of significance ( P = 0.05 ); however, the estimates of [d]', [h]' and $\delta_{[d]}$ are different for the two plasmatypes. This would suggest that there is a certain degree of plasmatic difference for the parentals M and C. The large $\delta_{[d]}$ estimates in both plasmatypes suggest that there may be gene dosis effects. The fact that the estimates are of different sign suggests that if gene dosis is present, it increases the expression of the paternal alleles when in homozygous state in ¶(M) but decreases the expression of paternal alleles when in homozygous state in ¶(C).

Table 13

a) Mean nitrogen content of the families having Inia ( I ) and Chinook ( CH ) as parents

| ¶( I ) families | ER | PR | ¶( CH ) families | ER | PR |
|---|---|---|---|---|---|
| I parent | 2.98 | 2.83 | CH parent | 2.98 | 2.84 |
| (ICH)F2 | 2.88 | 2.69 | (CHI)F2 | 2.82 | 2.69 |
| (ICH)F3 | 2.79 | 2.79 | (CHI)F3 | 2.82 | 2.86 |
| [I(ICH)]F2 | 2.81 | 2.86 | [CH(CHI)]F2 | 2.83 | 2.82 |
| [I(ICH)]F3 | 2.97 | 2.80 | [CH(CHI)]F3 | 2.78 | 2.72 |
| [I(CHI)]F2 | 2.89 | 2.91 | [CH(ICH)]F2 | 2.83 | 2.87 |
| [I(CHI)]F3 | 2.98 | 2.88 | [CH(ICH)]F3 | 2.90 | 2.81 |
| [(ICH)I]F2 | 2.86 | 2.90 | [(CHI)CH]F2 | 2.85 | 2.78 |
| [(ICH)I]F3 | 2.80 | 2.72 | [(CHI)CH]F3 | 2.87 | 2.78 |
| [(ICH)CH]F2 | 2.91 | 2.81 | [(CHI)I]F2 | 3.01 | 2.86 |
| [(ICH)CH]F3 | 2.90 | 2.92 | [(CHI)I]F3 | 3.06 | 3.11 |

b) Parameter estimates

| Parameters | ER ¶(I) | ER ¶(CH) | PR ¶(I) | PR ¶(CH) |
|---|---|---|---|---|
| $\hat{m}'$ | $2.93^{**} \pm .05$ | $3.04^{**} \pm .05$ | $2.93^{**} \pm .04$ | $3.02^{**} \pm .01$ |
| $[\hat{d}]'$ | $.02^{ns} \pm .05$ | $.13^{ns} \pm .06$ | $-.10^{ns} \pm .06$ | $.18^{*} \pm .02$ |
| $[\hat{h}]'$ | $-.18^{ns} \pm .17$ | $-.53^{ns} \pm .20$ | $-.45^{ns} \pm .14$ | $-.67^{**} \pm .05$ |
| $\hat{\delta}_{[d]}$ | --- | --- | $-.44^{ns} \pm .29$ | $.93^{*} \pm .10$ |
| $\hat{\delta}_{[h]}$ | --- | --- | $.58^{ns} \pm .22$ | $-.74^{*} \pm .08$ |
| $R^2$ | .31 | .68 | .90 | 1 |
| DF | 4 | 4 | 2 | 2 |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

I,CH Cross

The two lines, although having equal means at both locations, are genetically different and the genetic expression of the character is different in the two plasmatypes. This is especially noticeable at Parkland ( Table 13 ).

Although, in ¶(I) none of the genetic parameters other than the mid-parental value is significantly different from zero, the [h]', $\delta_{[d]}$ and $\delta_{[h]}$ estimates are large. The large $R^2$ value suggests that the lack of signifigance is due to reduced expression of the $U_k\{ch\}_k$ gene set in ¶(I). In ¶(CH) all the parameters are significant. The lines are different in additive gene effects and the alleles for lower nitrogen content are dominant. Dosis effects increase the expression of the character for homozygous loci but decrease it for heterozygous loci. It should be noted that the trends observed for dosis effects in ¶(I) are the reverse of those detected in ¶(CH).

No adequate model has been found for either plasmatype at Ellerslie. However, the fairly high $R^2$ value for ¶(CH) implies that the $U_k\{i\}_k$ gene set is not expressed in ¶(CH) and the mid-parental value m' is sufficient to describe the crosses. But this does not appear to be the case in ¶(I) where the best fitting model accounts for only 31% of the variation. The trend for ¶(CH) at Ellerslie does not appear to be similar to the model fitted at Parkland.

Table 14

a) Mean nitrogen content of the families having Marquis ( M ) and Inia ( I ) as parents

| ¶( M ) families | ER | PR | ¶( I ) families | ER | PR |
|---|---|---|---|---|---|
| M parent | 2.85 | 2.73 | I parent | 2.98 | 2.83 |
| (MI)F2 | 2.82 | 2.70 | (IM)F2 | 2.76 | 2.73 |
| (MI)F3 | 2.87 | 2.86 | (IM)F3 | 2.86 | 2.79 |
| [M(MI)]F2 | 2.73 | 2.80 | [I(IM)]F2 | 2.92 | 2.84 |
| [M(MI)]F3 | 2.87 | 2.77 | [I(IM)]F3 | 2.79 | 2.79 |
| [M(IM)]F2 | 2.75 | 2.78 | [I(MI)]F2 | 2.89 | 2.85 |
| [M(IM)]F3 | 2.94 | 2.96 | [I(MI)]F3 | 2.86 | 2.84 |
| [(MI)M]F2 | 2.79 | 2.77 | [(IM)I]F2 | 2.84 | 2.79 |
| [(MI)M]F3 | 2.74 | 2.82 | [(IM)I]F3 | 2.81 | 2.77 |
| [(MI)I]F2 | 2.81 | 2.89 | [(IM)M]F2 | 2.87 | 2.75 |
| [(MI)I]F3 | 2.83 | 2.94 | [(IM)M]F3 | 2.96 | 2.76 |

b) Parameter estimates

| Parameters | ER ¶(M) | ER ¶(I) | PR ¶(M) | PR ¶(I) |
|---|---|---|---|---|
| $\hat{m}$' | $2.85^{**}\pm.03$ | $2.95^{**}\pm.02$ | $2.97^{**}\pm.02$ | $2.80^{**}\pm.02$ |
| [$\hat{d}$]' | $0^{ns}\pm.03$ | $.04^{ns}\pm.03$ | $.24^{**}\pm.03$ | $.05^{ns}\pm.02$ |
| [$\hat{h}$]' | $-.08^{ns}\pm.11$ | $-.35^{*}\pm.07$ | $-.52^{**}\pm.06$ | $-.10^{ns}\pm.06$ |
| $\hat{\delta}_{[d]}$ | --- | $-.65^{ns}\pm.15$ | $-.33^{ns}\pm.13$ | --- |
| $\hat{\delta}_{[h]}$ | --- | $.28^{ns}\pm.12$ | $-.09^{ns}\pm.10$ | --- |
| $R^2$ | .11 | .97 | .99 | .78 |
| DF | 4 | 2 | 2 | 4 |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

M,I Cross

The $U_k\{m\}_k$ gene set in ¶(I), while not expressed at Parkland, is expressed at Ellerslie ( Table 14 ). While there is no evidence of a difference in additive gene effects for the two lines, they do differ genetically as is shown by the significant [h]' term at Ellerslie. The alleles for lower nitrogen content are dominant. The $\delta_{[d]}$ term, while large, is not significant; this would indicate a trend to reduced expression of the homozygous loci affecting the character in this location but no similar trend is observed at Parkland.

The $U_k\{i\}_k$ gene set is expressed at Parkland and the data can be described with an additive dominance model. The alleles producing lower nitrogen content are dominant.

No adequate genetic model has been found for ¶(M) at Ellerslie and no trends can be observed. The model with the lowest standard errors for the parameters accounts for only 11% of the variation in the data.

Table 15

a) Mean nitrogen content of the families having Ciano ( C ) and Chinook ( CH ) as parents

| ¶( C ) families | ER | PR | ¶( CH ) families | ER | PR |
|---|---|---|---|---|---|
| C parents | 3.39 | 3.15 | CH parent | 2.98 | 2.84 |
| (CCH)F2 | 2.93 | 2.87 | (CHC)F2 | 2.99 | 2.76 |
| (CCH)F3 | 3.02 | 3.02 | (CHC)F3 | 3.12 | 3.00 |
| [C(CCH)]F2 | 2.95 | 3.07 | [CH(CHC)]F2 | 2.99 | 3.04 |
| [C(CCH)]F3 | 3.31 | 3.20 | [CH(CHC)]F3 | 2.88 | 2.83 |
| [C(CHC)]F2 | 3.21 | 3.12 | [CH(CCH)]F2 | 3.14 | 3.00 |
| [C(CHC)]F3 | 3.10 | 3.04 | [CH(CCH)]F3 | 2.90 | 2.97 |
| [(CCH)C]F2 | 3.04 | 3.04 | [(CHC)CH]F2 | 2.99 | 2.96 |
| [(CCH)C]F3 | 3.24 | 3.00 | [(CHC)CH]F2 | 2.96 | 2.96 |
| [(CCH)CH]F2 | -- | 2.96 | [(CHC)C]F2 | 3.19 | 3.03 |
| [(CCH)CH]F3 | 2.95 | 3.04 | [(CHC)C]F2 | -- | 3.06 |

b) Parameter estimates

| Parameters | ER ¶(C) | ER ¶(CH) | PR ¶(C) | PR ¶(CH) |
|---|---|---|---|---|
| $\hat{m}'$ | $3.13^{**} \pm .01$ | $3.24^{**} \pm .01$ | $3.14^{**} \pm .02$ | $3.05^{**} \pm .07$ |
| $[\hat{d}]'$ | $.26^{*} \pm .02$ | $.26^{*} \pm .01$ | $.02^{ns} \pm .03$ | $.16^{ns} \pm .07$ |
| $[\hat{h}]'$ | $-.41^{ns} \pm .04$ | $-.51^{*} \pm .02$ | $-.51^{*} \pm .07$ | $-.42^{ns} \pm .25$ |
| $\hat{\delta}_{[d]}$ | $.41^{ns} \pm .09$ | $1.33^{*} \pm .04$ | $-.11^{ns} \pm .14$ | --- |
| $\hat{\delta}_{[h]}$ | $-.56^{ns} \pm .08$ | $-.89^{*} \pm .03$ | $.27^{ns} \pm .11$ | --- |
| $R^2$ | 1 | 1 | .98 | .58 |
| DF | 1 | 1 | 2 | 4 |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

C,CH Cross

The analysis ( Table 15 ) shows that the expression of the loci affecting the character nitrogen content is very similar for both plasmatypes at Ellerslie. All parameters are significant in ¶(CH) while only two parameters are significant in ¶(C) but the trend for the other three parameters in ¶(C) is the same as the results in ¶(CH); the observed discrepancies seem to be due to environmental variation within the location rather than to plasmatypic differences.

At Parkland the two plasmatypes are very different from each other and the genetic expression at this location is very different from Ellerslie. In ¶(C) only the [h]' term is significant and the alleles producing lower nitrogen content are dominant.

No adequate explanation has been found for ¶(CH) at Parkland. The fact that the model fitted accounts for 58% of the variation without any of the parameters ( except m' ) being significant suggests that the expression of the $U_k\{c\}_k$ gene set is reduced in ¶(CH).

Table 16

a) Mean nitrogen content of the families having Marquis ( M ) and Chinook ( CH ) as parents

| ♀( M ) families | ER | PR | ♀( CH ) families | ER | PR |
|---|---|---|---|---|---|
| M parent | 2.85 | 2.73 | CH parent | 2.98 | 2.84 |
| (MCH)F2 | 2.73 | 2.71 | (CHM)F2 | 2.62 | 2.79 |
| (MCH)F3 | 2.81 | 2.69 | (CHM)F3 | 2.93 | 2.77 |
| [M(MCH)]F2 | 2.95 | 2.83 | [CH(CHM)]F2 | 2.89 | 2.80 |
| [M(MCH)]F3 | 2.77 | 2.79 | [CH(CHM)]F3 | 2.84 | 2.82 |
| [M(CHM)]F2 | 2.85 | 2.75 | [CH(MCH)]F2 | 2.82 | 2.67 |
| [M(CHM)]F3 | 2.82 | 2.91 | [CH(MCH)]F3 | 2.86 | 2.90 |
| [(MCH)M]F2 | 2.71 | 2.77 | [(CHM)CH]F2 | 2.81 | 2.87 |
| [(MCH)M]F3 | 2.98 | 2.91 | [(CHM)CH]F3 | 2.77 | 2.73 |
| [(MCH)CH]F2 | 2.76 | 2.73 | [(CHM)M]F2 | 2.77 | 2.86 |
| [(MCH)CH]F3 | 2.80 | 2.92 | [(CHM)M]F3 | 2.98 | 2.75 |

b) Parameter estimates

| | ER | | PR | |
|---|---|---|---|---|
| Parameters | ♀(M) | ♀(CH)[a] | ♀(M)[a] | ♀(CH) |
| $\hat{m}'$ | $2.87^{**} \pm .01$ | $3.01^{**} \pm .05$ | $2.88^{**} \pm .06$ | $2.79^{**} \pm .03$ |
| $[\hat{d}]'$ | $.02^{ns} \pm .01$ | $-.03^{ns} \pm .08$ | $.04^{ns} \pm .06$ | $.05^{ns} \pm .04$ |
| $[\hat{h}]'$ | $-.27^{**} \pm .03$ | $-.73^{**} \pm .18$ | $-.35^{ns} \pm .22$ | $.01^{ns} \pm .11$ |
| $\hat{\delta}_{[d]}$ | $-.11^{ns} \pm .06$ | $-.73^{*} \pm .29$ | --- | $.34^{ns} \pm .22$ |
| $\hat{\delta}_{[h]}$ | $-.14^{ns} \pm .04$ | $.52^{ns} \pm .24$ | --- | $-.43^{ns} \pm .17$ |
| $R^2$ | .99 | .80 | .24 | .78 |
| DF | 2 | 6 | 8 | 2 |

( ** ) = $P \leq 0.01$

( * ) = $0.01 < P \leq 0.05$

( ns ) = $P > 0.05$

[a] backcross equations have not been pooled

M,CH Cross

The means of the derivatives of this cross and their analysis are in Table 16.

At Ellerslie, neither of the plasmatype sets of families have significant additive effects but both have significant dominance terms. The genetic expression of the character differs in the two plasmatypes as can be seen by comparing the [h]' terms ( $.46^{*} \pm .18$ ). Furthermore, the $\delta_{[d]}$ term is significant in ¶(CH) showing that gene dosis reduces the expression of the character in this plasmatype. There is no similar trend in ¶(M).

No adequate model has been found for either plasmatype at Parkland but the relatively high $R^2$ value for ¶(CH) suggests that the $U_k\{m\}_k$ gene set is not expressed in this plasmatype.

For two of the models lower standard errors result when the backcrosses are considered separately than when they are pooled. The trend for the results when the backcrosses have been pooled is the same as when they are considered separately; in the case of the ¶(CH) model at Ellerslie, the [h]' and $\delta_{[d]}$ terms are not significant when pooled backcrosses are used ( results not reported ).

## DISCUSSION

The degree of expression of the character nitrogen content, and by implication protein content, depends upon both the idiotype of the cultivar and the environment in which the cultivar is grown. In the present case, the variance component due to location effects is larger than that due to family effects in the analysis of variance of parentals, F2 and F3 generation. Clearly, environment was more important than idiotype in determining the expression of this character although the environmental conditions at the two locations where the experiment was conducted were not very different. More extreme variation on the expression of genetic potential for the four cultivars may be possible in other more diverse environments.

The results show that the effect of genotype on the expression of the character is not dramatic. However, this is not due to lack of sensitivity in the technique - micro-kjeldahl analysis is both sensitive and precise - nor to lack of material - twenty genetically different families are available for analysis of each pair of cultivars. On the other hand, the procedures of handling the materials available does place a limit on the types of genetic analysis which can be undertaken. For example, the seeds from different plants within a replication and from different replications within a location were pooled before performing the nitrogen determinations; thus, only the mean has been estimated and not its variance so that genetic analysis utilizing second degree statistics could not be employed for each location separately.

Since there are only four parents in this study, the validity

of the results of a diallel analysis ( Griffing 1956a,b, Mather & Jinks 1971 ) would be rather questionable. Nevertheless, Griffing's ( 1956b ) method of general and specific combining ability analysis has been used in this study by treating the locations as block replicates. Although this method has the advantage of simplicity, it amounts to little more than a set of descriptive statistics wherein the g.c.a. and s.c.a. estimates can be related to genetically meaningful parameters only under rather restrictive conditions ( Griffing 1956a ).

The results of the general and specific combining ability analysis show that the parentals differ with respect to both additive and non-additive gene action. It was found that additive gene action is the more important of the two in determining the level of expression of the trait. Furthermore, in the tables of specific combining abilities ( Tables 6 and 9 ) the diagonal terms are large and positive and the off-diagonal terms are small and of either sign. Negative heterosis was found in at least one generation at one of the locations for all crosses except M,C cross. In M,C cross, the values of the hybrids were between the mid-parental value and the mean for the lower parent. These results differ from those reported in a specific and general combining ability analysis of protein content in a nine parent diallel cross of Sorghum bicolor ( Collins & Pickett 1972 ). In sorghum the large s.c.a. estimates are found among the off-diagonal terms. Furthermore, in sorghum, positive heterosis was observed for two crosses involving inbred lines with higher than average protein content.

The families available limit the genetic analysis of means

since they do not permit [d] and $\Delta_{[d]}$ or [h] and $\Delta_{[h]}$ to be separated. The analysis can be performed using the parameters [d]' and [h]' but these confound additive and dominance effects, respectively, with the effect of the female plasmatype on the expression of the paternal gene set.

The analysis of means shows that there is idiotype-environment interaction affecting the level of nitrogen content; crosses involving different parents must be considered separately in the genetic analysis. While environment emerges as the single most important factor affecting the trait, plasmatype also effects the expression of the paternal set of alleles and must be taken into consideration in five of the six crosses analyzed. No consistent pattern can be observed for hybrid gene sets in different plasmatypes to gauge the relative importance of plasmatype in determining the expression of paternal alleles. Significant gene dosis effects were observed for several of the crosses but the expression of these effects seems to be complex depending both upon environment and plasmatype. A consisent observation is that all the [h] or [h]' terms significantly different from zero are negative implying that alleles for low nitrogen content are mostly dominant phenotypically. Similar results have been reported by Aksel ( 1956 ). in a study of several common wheat cultivars and their hybrids. In this latter study of parentals, F1 and F2 progeny, it was noted that most of the alleles effecting lower nitrogen content were dominant and in some cases there was negative heterosis.

Inverse correlations of yield with protein content have been reported by various authors for wheat and other cereal crops

( see Introduction ). Consequently, the mean yields ( Soomro 1974 ) for families from crosses involving M parent were averaged for the three replications within each location and a correlation analysis was performed for the two characters yield and nitrogen content. In contrast, no association was detected between the characters using either parametric ( $r = .12^{ns} \pm .09$ ) or non-parametric ( Kendall's $\tau = .058^{ns}$ $P = .17$ ) statistical methods. In most cases, the studies reported have been concerned with direct application of increasing protein yield while confounding the effects of genotype and environment on phenotype. It has been suggested ( Munck 1964 ) that the inverse correlation observed for yield and protein content in many studies does not imply genetic linkage or pleiotropic effects but reflects the effect of some limiting nutrient on the synthesis of protein in higher protein strains. In this study, the seeds were space-planted in experimental plots which had been fertilized with inorganic fertilizers before seeding. Consequently, the question of limiting nutrient effects can be ruled out and the plants should be able to express their full genetic potential. It is also possible that the correlation of the two characters is cross dependent and that, therefore, a correlation coefficient calculated on aggregated data from several different crosses is not meaningful. However, the pooling of seed samples within locations has greatly reduced the number of possible comparisons precluding attempts at detection of what may be weak associations for single crosses.

The character nitrogen content as measured here indirectly serves as a measure of protein content and, therefore, is of great

practical importance as a measure of flour quality or the nutritive value of grain for certain animals. Kjeldahl determination does not detect nitrogen in N-N, NO or $NO_2$ linkages ( A.O.A.C. 1955 ) but does detect nitrogen in C-N linkages. In wheat kernels, the majority of C-N linkages are in protein or free amino acid pools but a minor component is derived from nitrogen bases. It should be remembered that measuring the nitrogen content as grams nitrogen per grams dry-weight flour, expressed as a percent, does not, however, distinguish between the several possible causes of increase in the percent nitrogen. Desirable changes in certain cultivars such as an increase in the protein content resulting from an increased synthesis of protein cannot be distinguished from the situation in other lines in which there has been no change in the amount of protein synthesized but starch synthesis has been reduced. On the other hand, percent nitrogen is of great practical significance since most of the nitrogen is in endosperm and an increase can be correlated with several measures which are used to determine baking quality ( Pomeranz 1971 ). Finally, although the method of nitrogen determination used here does not distinguish changes among different tissues in the wheat kernel, this would make little difference where whole seeds are used as, for example, in animal nutrition.

The results of this study imply that the simplist way of increasing the protein content of wheat is by manipulating the environment. For example, heavy nitrogen fertilization will increase the protein content of cereals and reduce the amount of protein supplement necessary for balanced nutrition. Since additive genetic effects are

more important than non-additive genetic effects for the character nitrogen content, it should be possible to select homozygous wheat lines with the genotype and plasmatype necessary for high protein content. Furthermore, the present lack of correlation of nitrogen content with yield would imply that some gains in yield could be selected for as well. However, it is clear that these lines would also have to possess other desirable characteristics like earliness and disease resistance. Should there be negative correlations between these characters and nitrogen content, it would be more difficult to incorporate all of these necessary characters in the same line. Finally, taking economic factors into consideration, it may well prove more profitable to supplement cereals with high protein plant and animal products than to attempt direct genetic improvement of the protein content of cereals.

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## APPENDIX I

Mean percent nitrogen values for the five parental cultivars in each replication

| | Ellerslie | | | Parkland | | |
|---|---|---|---|---|---|---|
| Cultivars | 1 | 2 | 3 | 1 | 2 | 3 |
| M | 2.790 | 2.838 | 2.895 | 2.680 | 2.727 | 2.595 |
| K | 2.760 | 2.568 | 2.890 | 2.462 | 2.580 | 2.470 |
| C | 3.322 | 3.214 | 3.505 | 3.119 | 3.050 | 3.140 |
| I | 3.005 | 2.910 | 3.035 | 2.645 | 2.785 | 2.770 |
| CH | 2.885 | 2.940 | 3.055 | 2.885 | 2.695 | 2.810 |

## APPENDIX II

Mean percent nitrogen for the F2 and F3 generation of the 4x4 diallel

F2 generation

| | Ellerslie | | | | | Parkland | | | |
|---|---|---|---|---|---|---|---|---|---|
| | M | C | I | CH | | M | C | I | CH |
| M | 2.863 | 3.147 | 2.820 | 2.730 | M | 2.743 | 2.900 | 2.700 | 2.71 |
| C | 2.910 | 3.383 | 2.930 | 2.930 | C | 2.920 | 3.153 | 2.900 | 2.850 |
| I | 2.760 | 2.930 | 2.980 | 2.880 | I | 2.730 | 2.940 | 2.830 | 2.690 |
| CH | 2.620 | 2.990 | 2.820 | 2.973 | CH | 2.790 | 2.760 | 2.690 | 2.840 |

F3 generation

| | Ellerslie | | | | | Parkland | | | |
|---|---|---|---|---|---|---|---|---|---|
| | M | C | I | CH | | M | C | I | CH |
| M | 2.865 | 3.053 | 2.870 | 2.810 | M | 2.743 | 2.786 | 2.860 | 2.690 |
| C | 3.020* | 3.383 | 3.030 | 3.020 | C | 2.933 | 3.153 | 2.960 | 3.023 |
| I | 2.860 | 2.980 | 2.980 | 2.790 | I | 2.790 | 2.970 | 2.830 | 2.790 |
| CH | 2.930 | 3.120 | 2.820 | 2.973 | CH | 2.770 | 2.993 | 2.857 | 2.840 |

Means over the two locations

| | Ellerslie | | | | | Parkland | | | |
|---|---|---|---|---|---|---|---|---|---|
| | M | C | I | CH | | M | C | I | CH |
| M | 2.803 | 3.024 | 2.760 | 2.720 | M | 2.803 | 2.920 | 2.865 | 2.750 |
| C | 2.915 | 3.268 | 2.915 | 2.890 | C | 2.981 | 3.268 | 2.995 | 3.022 |
| I | 2.745 | 2.935 | 2.905 | 2.785 | I | 2.825 | 2.975 | 2.905 | 2.790 |
| CH | 2.705 | 2.875 | 2.755 | 2.907 | CH | 2.850 | 3.057 | 2.839 | 2.907 |

*missing value estimated by the least squares technique